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Print Form

This report is required by law (? USC 21 43) Failure to report according to the regulations can result in an order to cease and desist and to be subject lo penalties as provided for in Section 2150

Set reverse side for additional information

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. **REGISTRATION NO.** 86-R-0003

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA include Zip Code)

University of Arizona
University Animal Care\1501 N. Campbell Ave.,Rm. 1126
Tucson, AZ 85724
Telephone: (520)626-6702

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites)

See attached listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
4. Dogs			26		26
5. Cats		8	11		19
6. Guinea Pigs	7	161			~~168~~ 161
7. Hamsters		42	25		67
8. Rabbits	9	2	134		~~145~~ 136
9. Non-human Primates		1	10	1	12
10. Sheep			30		30
11., Pigs	2		241	27	~~270~~ 268
12. Other Farm Animals cattle			17		17
Goats			4		4
13 Other Animals squirrels		119			119
Skunks		177			177
Bat		80			80
Coyotes		8			8
Mt. Lion		10			10

Per phone 11-28-06

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary **of all such exceptions is attached lo this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional Official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

SIGNATURE

[redacted] b6, b7C

DATE SIGNED

11/22/06

APHIS FORM 7023 (AUG 91)

(Replaces VS FORM 18-23 (OCT 88) which is obsolete

NOV 27 2006 ✓

Column E Explanation: Pigs

1. **Registration Number**: 86-R-0003

2. **Number of Animals used in the study:** 27

3. **Species:** Pig

4. **Procedure:** There are two research projects, funded by USDA and being conducted by the same research group, with the aims of identifying genes expressed in *Campylobacter jejuni* virulence. While the main thrust of this research is for the hog industry, the results would be applicable to any species, including humans that contract Campylobacteriosis. The studies are evaluating pathogenicity, utilizing various mutants of the organism. The studies should confirm that mutations in genes involved in macrophage survival are directly related to the ability of the isolate to produce lesions in the host.

   Newborn piglets are fasted for 6 hours and then inoculated with *Camphylobacter jejuni* transformants. Fecal samples are taken before inoculation and during the evaluation period. All piglets are observed daily for clinical signs of campylobacteriosis (diarrhea). Fecal samples are collected daily for the identification of *C. jejuni* DNA by PCR. Depending upon clinical signs and shedding of the microorganism, piglets are euthanized at 9, 48, and 120 hours post-inoculation and necropsied. Small and large intestinal tissue is collected in the vicinity of areas displaying gross lesions for microscopic examination. Any animal that is unable to feed or water itself or unable to rise without aid is immediately euthanized.

5. **Justification for withholding pain/distress aids:** The administration of analgesics, etc, may affect the normal progression of disease and microscopic lesions necessary for diagnosis. It has been shown (Kohn and Muir, 1996) that the use of opioid analgesics, such as methadone, can release histamines, decrease gut motility, etc. NSAIDs inhibit the inflammatory response. Neostigmine and xylazine decrease gut motility (Kohn and Muir, 1988). Gut motility and host inflammatory response are critical to the progression of disease or lack thereof. It would be contradictory to the study to introduce compounds that would effect these factors, whether individually given or in conjunction. The scope of the project is to reproduce disease as seen in the "field" and examine protective measures, not to investigate the effects of various analgesics on establishment of disease. The clinical signs observe–watery/bloody diarrhea, diminished appetite, mild weight loss–are observed in the normal progression of the disease. All moribund animals are immediately euthanized.

6. **Federal Regulations requirements:** None

Column E Explanation: NHP

1. **Registration Number**: 86-R-0003

2. **Number of Animals used in the study:** 1

3. **Species:** M. mulatta

4. **Procedure:** This study involved the use of MECS to induce gene stimulation in the brain. The study was funded by the National Institute on Aging of the National Institutes of Health. The University of Arizona Institutional Animal Care and Use Committee, university veterinarians, principal investigator, external and internal reviewers, and others spent countless hours on the review and revision of this study to ensure that standards for humane animal care and use promulgated by the Animal Welfare Act and Public Health Service Policy on the Humane Care and Use of Laboratory Animals are met.

The procedure was done under the following anesthesia, analgesia, and euthanasia procedures, which were obtained from the leading MECS research in the US. The researcher who provided the protocol regimen came to the University of Arizona and actually performed the procedure for the UA researcher in accordance with UA IACUC requirements.

A. Initial anesthesia for transport: 10 mg/kg Ketamine given intramuscularly
B. Anesthesia during ECT (MECS): Methohexital bolus of 1 mg/kg followed by succinylcholine at 3.5 mg/kg IV bolus.
C. Atropine at .5 mg/kg administered to reduce seizure-induced secretions and to protect the airway.
D. Immediately after seizure is induced, the animal will be given an intra-thoracic injection of beuthanasia at 1 cc/10 lbs body weight.

The MECS procedure, if performed without the blocking agent would cause extreme pain and distress due to violent contractions of the muscles with strong potential for broken bones of the animal and potential injury to humans in the vicinity of the animals. A university neurologist provided the following statement to the IACUC: "succinylcholine serves as an analgesic in this procedure by blocking the muscular contractions, which would cause pain during MECS.

The following physiological monitoring was performed continuously during the procedure through the following methods:

The animal was placed on continuous positive pressure 100% oxygen during the procedure. The animal was monitored through pulse-oximetry, end-tidal PCO_2 and blood pressure. A veterinarian was present throughout the procedure to participate in the monitoring and did not indicate any signs of pain or distress, as observed in monitoring

data and observation of the animal.

5. **Justification for withholding pain/distress aids:** As noted above this procedure was performed with anesthesia and analgesia, but an original categorization of this protocol as an E remained due to concerns prior to the actual performance of the procedure. There were lingering concerns on the part of a quorum of the IACUC to keep this as an "E" procedure. The use of only one animal was approved and the veterinarian's follow-up statement after the procedure was placed in the record to show that there was no evidence of pain and distress during the procedure. Original concerns of the IACUC were that the dosage of Methohexital was lower than the full anesthetic dose quoted in the veterinary literature. There was a considerable amount of scientific literature utilizing the MECS procedure in animals and not indicating pain or distress. The IACUC wanted more assurance. A university neurologist, who performs MECS in human patients provided the following information: He indicated that the full anesthesia dose of Methohexital is the same as the monkey full anesthetic dose (6-10 mg/kg). The dose in humans for MECS is 1 mg/kg + 2.5 mg kg succinylcholine. The person is first given Ketamine, then succinylcholine is given next, followed by methohexital If given alone, the 1 mg/kg Methohexital would provide more than sedation, but is a subanesthetic dose. The reason it is given subanesthetic, is that if the human patient was fully anesthetized, then the MECS procedure could not be controlled and the electroshock would be "uncontrolled", meaning that too much of the brain might be mistakenly stimulated causing damage to the brain and the person once they recover. Additionally, traditional anesthesia would block the seizure response, thus negating the treatment. Patients, who have received 1 mg/kg of Methohexital, the same as our monkeys would receive, do not remember experiencing any pain and do not complain about pain following the ECT procedure. Human patients are not afraid to have the procedure performed again. He also discussed the pain that would be experienced if the person was awake and had this procedure done. Direct cortical stimulation does not produce pain—the patient does not feel cerebral pain and cortical stimulation leading to muscle movement does not cause muscle pain. Epileptics do not experience muscle pain from the jerking movements. The only time nerve pain is felt is when there is peripheral nerve stimulation, which does not occur in MECS/ECT. The pain that is experienced from MECS is when there are violent muscle contractions, which breaks bones—then there is the pain of broken bones. By giving a human or animal succinylcholine, the muscle contractions that could lead to broken bones are prevented; thus, succinylcholine is serving as the pain blocker for this procedure.

Additionally, the IACUC had concerns with training, as the researchers at the UA had not performed this procedure before in NHP, although they had performed many similar procedures in rats. The IACUC Chair contacted the leading US researcher who does MECS. She (researcher) provided the most up-to-date anesthetic regimen, based on her latest research. She also indicated that training is of the utmost importance in assuring the success of the procedure and ensuring animals will not suffer pain and/or distress. Because of the training issue, the IACUC required that this leading researcher come to the UA and perform the procedure. She came and did the procedure, while UA researchers

and veterinarians observed.

6. **Federal Regulations requirements:** None

The attending veterinarian stayed in contact with USDA and OLAW staff members throughout the development and conduct of this experiment. Additional information can be provided upon USDA request.